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6-Thioguanine Resistant Peripheral Blood Lymphocytes in Humans Following Psoralen, Long-Wave Ultraviolet Light (PUVA) Therapy

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A recently described method that enumerates variant 6-thioguanine resistant peripheral blood lymphocytes present *in vivo* in man as a potential marker of somatic cell mutations occurring *in vivo* was used to study 18 psoriatic patients receiving PUVA therapy, 16 conventionally treated psoriatic patients, 10 vitiligo patients receiving PUVA therapy and 7 untreated individuals with vitiligo. Variant lymphocyte frequencies determined for these individuals were compared with those determined for groups of 10 concurrent and 63 cumulative healthy control individuals. Variant frequencies were elevated in psoriatic and vitiligo patients receiving PUVA therapy and in conventionally-treated psoriatic patients. They were not elevated over control values in untreated vitiligo patients.

There is some uncertainty as to what constitutes the best approach to the controlled use of mutagenic agents with the potential for human benefit [1–3]. Effective medical therapies pose a particularly difficult problem in this regard. For this reason, the ability to assess genetic damage resulting from such agents under the conditions of exposure in terms relevant to man is of critical importance.

It is well established that psoriasis often responds dramatically to photochemotherapy with one of a variety of psoralen compounds and long-wave ultraviolet light (PUVA) [4,5]. Psoralens covalently bind to thymidine in DNA to form monofunctional or bifunctional photoadducts with interstrand crosslinks [6–10]. Although this activity probably accounts for PUVA's effectiveness, it has been suggested that it may also have genetic or oncogenic consequences [5].

Naturally occurring germinal mutations in man of the X-chromosomal gene for hypoxanthine-guanine phosphorbosyltransferase (HG-PRT) give rise to the well characterized Lesch-Nyhan (LN) Syndrome [31,32]. All somatic cells from LN males have the mutant gene, are deficient in HG-PRT, are unable to phosphorylate purine analogues and thus are resistant to the cytotoxicity of these drugs [22,23]. These cells provide prototypes at the single cell level for this mutant phenotype. Because of random X-chomosomal inactivation, [33,34] some somatic cells from pheno-typically normal females heterozygous for the LN mutation are also mutant [35,36]. The detection of purine analogue resistant cells in heterozygotes provides a model for cellular mosaicism for this genetic resistance marker. The naturally occurring LN mutation was used to develop and calibrate the system used to detect somatic cell mutants arising in non-LN individuals in the present studies (21).*

Earlier, one of us (RJA) had co-developed the human diploid fibroblast system, also modeled on the behavior of LN prototype mutant cells, to detect environmental agents deleterious to the human genetic material *in vitro* [37]. The fibroblast system has since been used to define the mutagenicity of several environmental agents—including known carcinogens—for human cells [38,39], has detected the mutagenicity of PUVA when applied to human fibroblasts *in vitro* [15] and has demonstrated the extreme sensitivity of fibroblasts from individuals with Xeroderma Pigmentosum to ultraviolet light induced mutation [40]. The rationale for using the purine analogue resistance marker for studies of somatic cell mutation is presented elsewhere [21,37].*

We have found that 6-thioguanine resistant (TG^r) peripheral blood lymphocytes (PBL's) are present in low frequencies in the blood of healthy, non-LN individuals, and that these frequencies are elevated in groups of cancer patients treated with chemotheraputic agents which are known mutagens [21].* In the current study we determined the frequency of variant TG^r PBL's (variant frequency = V_f) for 2 groups of psoriatic patients—those receiving conventional and those receiving PUVA therapy. We did likewise for a group of untreated vitiligo patients and for a group receiving PUVA therapy. We compared these V_f's with those determined for healthy controls. We now report these results.

MATERIALS AND METHODS

The method of the TG^r PBL autoradiographic assay has been described in detail [21].

Lymphocyte Culture

Venous blood is collected in a heparinized syringe (Beef lung; Upjohn 1000 units/ml, 0.1 ml/10 ml blood) and mixed with an equal volume of Hank's Balanced Salt Solution (HBSS) containing 0.2% (v:v) heparin and 2% (v:v) penicillin/streptomycin mixture (100 units/ml and 100 μg/ml respectively; Gibco). Ficoll-Hypaque (2.4 parts of 9% Ficoll and 1 part 34% Hypaque) is carefully underlayed, and the mixture centrifuged at 600 ×*g* for 30 min. The white blood cell layer is removed, washed ×2 with supplemented HBSS and resuspended in complete medium containing 80 volumes RPMI 1640 with 24 mM Hepes buffer (Microbiological Associates), 0.2% heparin, 2% penicillin/streptomycin, and 20 volumes human AB plasma previously heat-inactivated at 56°C for 20

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Abbreviations:
HG-PRT = Hypoxanthine-guanine phosphorbosyltransferase
LN = Lesch-Nyhan
PBL = Peripheral blood lymphocyte
SCE = Sister chromatid exchange
TG^r = Thioguaine resistant
V_f = Variant frequency

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min and centrifuged at 6000 ×*g* for 10 min. Cell suspensions are made to contain 1.1×10^6 cells and 25 μg reconstituted Bacto-Phytohemagglutinin (PHA; Gibco), per ml. 0.1 ml of TG solution or pH adjusted water (control), is added to 0.9 ml of cell suspensions. Triplicate 1 ml cultures are incubated in a humidified 5% CO_2 atmosphere at 37°C for 24 hr in 16 × 100 ml screw-cap glass point-bottom culture tubes (Bellco) followed by the addition of 1 microcurie ^{3}HTdr (S.A. 2.1; New England Nuclear) and an additional 6 hr incubation.

Autoradiography of Free Nuclei

The method for preparation of free nuclei is a modification of that of Sanford et al [41]. At termination of culture, 4 volumes of 0.1 M citric acid at 4°C are added to tubes which are centrifuged at 600 ×*g* for 10 min and the supernatant removed. The pellets are resuspended in methanol:acetic acid (5:1) fixative, centrifuged again at 600 ×*g* for 10 min and the supernatant removed. The pellets are now resuspended in 0.2 ml fixative, left to fix at 4°C for at least 3 hr, and triturated through a 25 gauge spinal needle. The free nuclei in suspension are enumerated by counting a 0.025 ml aliquot with an electronic particle counter (Coulter Electronics, ZBI Model). The remaining nuclei in suspension are carefully added to one of three 18 × 18 mm coverslips which are affixed with Permount (Fisher Scientific) to glass microscope slides. Dried slides are stained with 1% aceto-orcein for 2 min and rinsed with distilled water.

To autoradiograph, the dried slides are mechanically dipped into NTB-2 (Eastman Kodak) emulsion pre-warmed to 43°C, exposed in dark boxes at −70°C for 1–2 weeks, developed in D19 (Eastman Kodak) developer at 17°C for 4 min, stopped with a 1 minute exposure to 1% acetic acid and fixed in "hypo" (Eastman Kodak) for 5 min [42]. Slides are then rinsed in distilled water for 1 min and air-dried. Later studies showed that slides could be exposed at −12°C for periods as short as 24 hr.

Counting

Twenty-five hundred nuclei on slides from PHA cultures not containing TG are counted by high-power light microscopy. The incidence of autoradiographically labelled cells is determined as the labelling index (LI). All nuclei on slides prepared from PHA stimulated cultures containing TG are viewed by low-power (100×) microscopy and all labeled nuclei on the slides are counted. The LI for TG containing cultures is determined from the total number of nuclei on the slides as enumerated by the Coulter counter. The V_f is then determined as the LI of PHA stimulated cultures containing TG divided by the LI of PHA stimulated cultures not containing TG as follows:

$$\frac{\text{LI(PHA + TG)}}{\text{LI(PHA)}} = V_f$$

Solutions

6-Thioguanine (TG) (2-amino-6-mercaptopurine; Sigma) solution was made as a 2×10^{-2} M stock, sterilized by passage through a 4 μ filter (Millipore) and added to pH adjusted water in appropriate amounts to make test concentrations. Aceto-orcein stain was obtained from Gibco.

Human Subjects

Healthy individuals of the cumulative control group were obtained from laboratory personnel, staff of the Medical Center Hospital of Vermont (MCHV) and other volunteers. Patients with psoriasis were under the care of the Dermatology Unit, Department of Medicine, University of Vermont College of Medicine (PAK) or the Dermatology Unit, Hitchcock Clinic and Dartmouth Medical School (RBD). PUVA therapy for patients with psoriasis was given at Hitchcock Clinic, Dartmouth Medical School. Blood samples from patients with vitiligo were kindly provided by Dr. David Mosher, Massachusetts General Hospital where vitiligo patients received PUVA therapy. Blood samples were obtained after receiving informed consent.

Characteristics of Study Groups

Concurrent controls: This group consisted of 10 healthy individuals, (5 women and 5 men) with an average age of 34 yr (range 21–58).

Psoriatic Patients

a. Conventional therapy group: Sixteen psoriatic patients (9 women and 7 men) with an average age of 55 yr (range 24–86) were undergoing conventional therapies consisting of topical steroids (14 patients), modified Goeckerman technique (4 patients), modified Ingram technique (2 patients), UVB without tar (3 patients), and methotrexate (2 patients). Skin types included I–IV (43); % of body involved varied from 5–90%.

b. PUVA therapy group: Eighteen psoriatic patients (5 women, 13 men) with an average age of 50 yr (range 31–68) were receiving PUVA treatments with 8 methoxypsoralen and UVA (total dosage 289 jules/cm^2 to 4504 jules/cm^2). All had received conventional therapy and at least 5 of these 18 had had methotrexate at some time in the past. Every patient had at least 26 PUVA treatments. Skin types included I–IV; % of body involved at time of last treatment varied for 1% to 15%.

Vitiligo Patients

Untreated group: Seven vitiligo patients (4 women, 3 men) with an average age of 31 yr (range 14–57) were not on treatment. Three of these 7 had received psoralen and sun treatment at some time in the past. These 7 patients had from 5 to 35% body involvement.

PUVA therapy group: Ten vitiligo patients (3 women, 7 men) with an average age of 27.5 yr (range 4–47) were receiving PUVA treatment with 8-methoxypsoralen (4 patients) or trimethoxypsoralen (6 patients) and UVA (total dosage 635 joules/cm^2 to 3950 joules/cm^2). The number of PUVA treatments for this group ranged from 12 to 320. These ten patients had from 5 to 70% body involvement.

Statistical Analysis

Statistical evaluations of differences in the distributions of V_f values between groups were made with the Wilcoxon Two Sample Test. p-values determined in this way were unadjusted. A more conservative estimate of the significance of V_f differences among groups when compared simultaneously was made with the Kruskal-Wallis test for 6 populations (cumulative controls, concurrent controls, PUVA treated psoriatics, conventionally-treated psoriatics, PUVA treated vitiligo patients and untreated vitiligo patients).

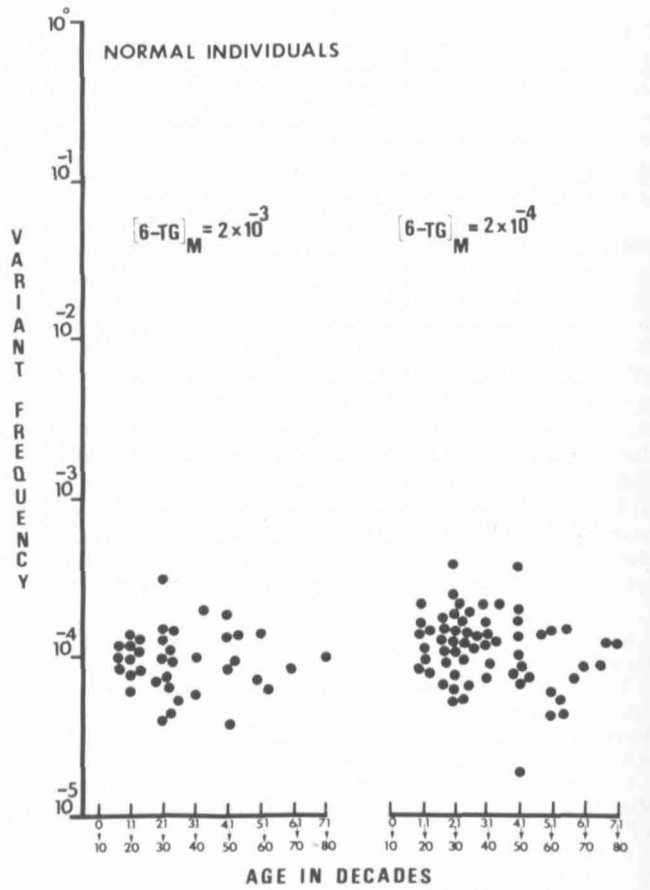

FIG 1. Frequencies of TG^r PBL's (V_f's) in healthy non-LN individuals shown by age of the individual. The *ordinate* shows V_f on a $\log_{10}$ scale; the *abcissa* depicts age by decade. TG^r of PBL's was determined at 2×10^{-3} M and 2×10^{-4} M TG.

RESULTS

PBL V_f's in non-LN Healthy Individuals

TG^r PBL frequencies were determined for 83 healthy individuals, some of whom served as concurrent controls for this study. In the entire cumulative control groups, 71 experiments in 38 individuals determined V_f's at 2×10^{-3} M TG and 98 experiments in 63 individuals determined V_f's at 2×10^{-4} M TG. Eighteen individuals were tested at both TG concentrations. The age of individuals ranged from 11 to 75 yr. The median V_f when determined at 2×10^{-3} M TG was 1.0×10^{-4} (mean = 1.06×10^{-4}) (10th and 90th percentile = 5×10^{-5} and 1.5×10^{-4}) and, when determined at 2×10^{-4} M TG, was 1.1×10^{-4} (mean = 1.3×10^{-4}) (10th and 90th percentile = 6.1×10^{-5} and 2.1×10^{-4}).

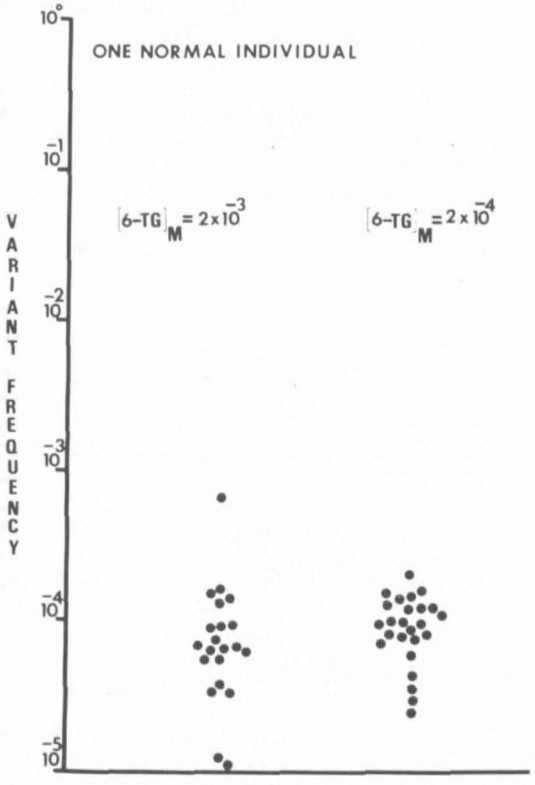

FIG 2. Frequencies of TG^r PBL's (V_f's) of a single healthy non-LN individual (ordinate, $\log_{10}$) determined over a 2-yr time interval. TG^r of PBL's was determined at 2×10^{-3} and 2×10^{-4} M TG.

Figure 1 gives the results of these experiments with PBL V_f's arranged according to age of sample donor. The *ordinate* shows V_f's on a $\log_{10}$ scale; the *abcissa* gives age by decade. Each *point* represents one, or the average of several, tests with cells from one individual. As can be seen, there is no apparent correlation of V_f with age.

Figure 2 presents the results of multiple tests over a 2-yr time interval with PBL's from a single individual. Again, V_f's are given on the ordinate ($\log_{10}$). Twenty-one experiments tested cells from this individual at 2×10^{-3} M TG, while 24 tested cells at 2×10^{-4} M TG. At the higher concentration, the range of V_f's was 6.2×10^{-4} to 1×10^{-5} while at the lower concentration it was 2×10^{-4} to 3.1×10^{-5}. Although the former represents a 60-fold range, the value of 6.2×10^{-4} is the highest we have ever determined in a healthy control, and this on only a single occasion.

PBL V_f's in Patients With Psoriasis

TG^r PBL V_f's were determined at 2×10^{-4} M TG for 16 psoriatic patients undergoing conventional therapy and 18 patients receiving PUVA. All PUVA treated patients had received at least 26 treatments at the time of the test.

The Table 1 presents numerical results from a sample experiment. In this experiment, V_f's were determined for a single concurrent control individual, and for 3 psoriatic patients receiving PUVA treatments. The labeling indices ranged from 0.022 to 0.070 and the psoriatics and controls were not different in this regard. The TG^r PBL V_f of the control was 9.8×10^{-5} while, for the 3 PUVA treated psoriatic patients, the V_f's were 6.4×10^{-3}, 7.4×10^{-4} and 6.7×10^{-4}. It can be seen that the absolute number of TG^r variant cells as determined from autoradiographically-labeled nuclei in the PHA + TG cultures as well as the V_f's were elevated for the PUVA treated psoriatics.

All of the V_f values determined in many similar experiments with PBL's from psoriatic patients and concurrent controls are shown in Fig 3 where V_f's are indicated on the ordinate ($\log_{10}$) and columns represent different groups of individuals. The wavy lines represent the median and 10th and 90th percentile V_f values determined at 2×10^{-4} TG for the cumulative

TABLE I. *PBL V_f's Determined at 2×10^{-4} M TG in Psoriatic Patients Receiving PUVA Therapy*

Individual	PHA		Cultures incubated with PHA + TG 2×10^{-4}M				LI (PHA+TG)
	LI[a]	Avg. LI	# labeled nuclei/slide[b]	# nuclei slide $\times 10^{6c}$	LI[a]	Avg LI	LI(PHA) $=V_fTG^{rd}$
Normal control	.040	.042	2	.530	3.8×10^{-6}	4.1×10^{-6}	9.8×10^{-5}
	.044		2	.470	4.3×10^{-6}		
Psoriatic Patient A PUVA treated	.024	.022	73	.522	1.4×10^{-3}	1.4×10^{-3}	6.4×10^{-3}
	.020		81	.580	1.4×10^{-3}		
Psoriatic Patient B PUVA treated	.075	.070	21	.453	4.6×10^{-5}	5.2×10^{-5}	7.4×10^{-4}
	.065		27	.467	5.8×10^{-5}		
Psoriatic Patient C PUVA treated	.058	.060	33	.765	4.3×10^{-5}	4.0×10^{-5}	6.7×10^{-4}
	.062		27	.723	3.7×10^{-5}		

[a] = Labeling index.
[b] = Determined by count of entire slide.
[c] = Determined by Coulter count of nuclei in suspension.
[d] = Variant frequency.

V_f's are shown for a single control individual and for 3 PUVA treated psoriatic patients. The labeling index for the control is 0.042; for the PUVA treated psoriatic patients the indices are 0.022, 0.070 and 0.060. The TG^r PBL V_f of the control is 9.8×10^{-5} and absolute numbers of autoradiographically labeled nuclei counted in replicate PHA+TG cultures are 2, and 2. The TG^r PBL V_f's of the PUVA treated patients are 6.4×10^{-3}, 7.4×10^{-4} and 6.7×10^{-4} based on absolute autoradiographically labeled nuclei replicate counts of 73, 81; 21, 27 and 33, 27 respectively (see methods).

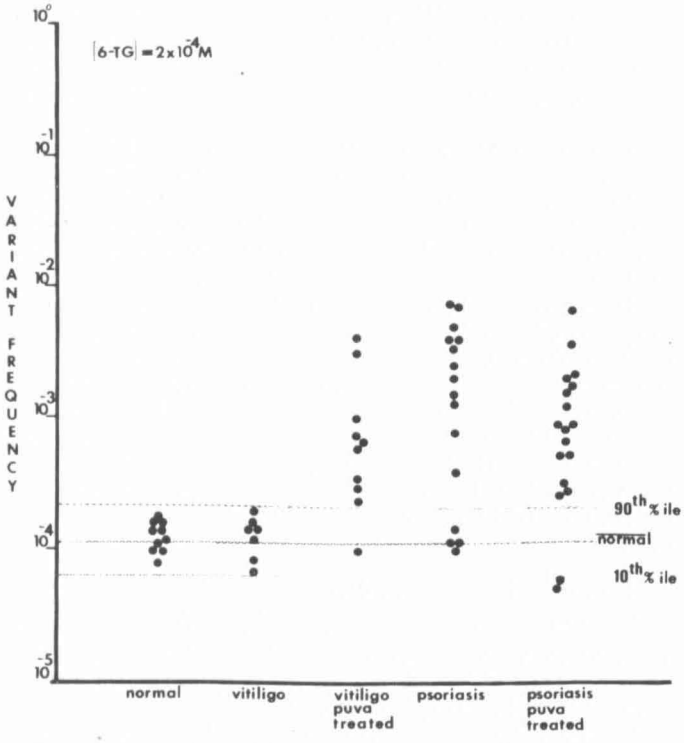

FIG 3. Frequencies of TG^r PBL's (V_f's) (*ordinate*, $\log_{10}$) are shown for psoriatic patients receiving conventional therapy, for psoratic patients receiving PUVA therapy, for untreated vitiligo patients, for vitiligo patients receiving PUVA therapy and for healthy concurrent controls. The *wavy lines* represent the median TG^r PBL V_f values of a group of 63 healthy individuals tested also at 2×10^{-4} M TG. The 10th and 90th percentiles for these control values are presented. Sixteen of 18 PUVA treated psoriatics, 12 of 16 conventionally treated psoriatics and 9 of 10 PUVA treated vitiligo patients have TG^r PBL V_f values above the 90th percentile of the cumulative control group values. By contrast, no concurrent control and no untreated vitiligo patients have V_f values greater than the 90th percentile value of the cumulative controls.

control group. Concurrent controls in these experiments served as methodological controls. Although quite small, the distribution of V_f's of the concurrent control group does not differ from the distribution found in the cumulative control group, of which it is a part. By contrast, psoriatic patients receiving PUVA treatments present a distribution of V_f values which shows them to be significantly greater than those of the cumulative control group ($p < 0.001$; Wilcoxon's Two-Sample Test). The median V_f of PUVA treated patients is 8.0×10^{-4} (mean = 1.2×10^{-3})(10th and 90th percentile = 5.9×10^{-5} and 3.5×10^{-3}). Likewise, psoriatic patients receiving conventional therapy present TG^r PBL V_f elevations which are at least as great as those that are seen in patients receiving PUVA. For the conventional therapy psoriatic group, the median V_f is 1.8×10^{-3} (mean = 2.6×10^{-3})(10th and 90th percentile = 1.2×10^{-4} and 7.5×10^{-3}). The distribution of V_f values is not different between this group and the PUVA treated psoriatics while it is significantly different between them and the cumulative control group ($p < 0.001$ Wilcoxon's Two-Sample Test). Even when simultaneous group comparisons of V_f values are evaluated, both PUVA and conventionally treated psoriatic V_f's are significantly elevated over cumulative control group values ($p < 0.05$). When compared with the smaller concurrent control group, conventionally treated psoriatics' V_f elevations over controls are significant while those of the PUVA treated group approach significance at an alpha level of 0.05.

PBL V_f's in Patients with Vitiligo

Because of the difficulty of finding untreated psoriatic patients, a group of individuals was sought who would be either untreated or receiving PUVA therapy. Therefore, TG^r PBL V_f's were determined at 2×10^{-4} m TG for 7 untreated and 10 PUVA treated vitiligo patients. The results of these studies are also shown on Fig 3. As can be seen, vitiligo patients receiving PUVA therapy have elevated TG^r PBL V_f's when compared with normals with a median value of 5.1×10^{-4} (mean = 1.1×10^{-3})(10th and 90th percentile = 9.6×10^{-5} and 4.2×10^{-3}). The V_f values in this group are significantly greater than those of the cumulative control group ($p < 0.005$) and even approach a significant elevation at this alpha level over the small concurrent control group. By contrast, for untreated vitiligo patients, the group median TG^r PBL V_f value is only 1.4×10^{-4}. Seven individuals comprise this group, and this small number is insufficient to demonstrate a statistical difference in V_f values between them and the PUVA treated vitiligo group. However, 9 of the 10 V_f values for PUVA treated vitiligo patients are greater than any in the non-PUVA treated group. All V_f values for the nontreated vitiligo group fall with the 10th and 90th percentile for the V_f values of the cumulative control group while 9 of the 10 V_f values for PUVA treated vitiligo patients are above the 90th percentile for cumulative controls.

DISCUSSION

We have shown both here and elsewhere that TG^r PBL's occur in normal individuals at low frequencies which are elevated in persons exposed to putative mutagens. The TG resistant phenotype of these cells resembles that of LN PBL's where an X-chromosomal gene mutation results in deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HG-PRT)[22,23,31,32]. This enzyme activity is necessary to phosphorylate purine analogues and render them cytotoxic [26–30]. A similar resistance, which is also associated with HG-PRT deficiency, can be induced in normal fibroblast exposed *in vitro* to mutagens [37]. This resistant phenotype is heritable over the somatic line in fibroblasts and is assumed to result from a process akin to mutation. The fibroblast system has been used to detect agents which damage the human genetic material [15,38–40].

Although we do not know the nature of TG^r PBL's in non-LN individuals, it seems reasonable to assume that at least some of them also result from somatic cell mutation. Furthermore, the X-chromosomal location of the gene for HG-PRT makes mutation at this locus an appealing explanation for TG resistance because expression of the resistant phenotype should follow directly from mutation. The observed frequencies of TG^r PBL's are consistent with mutation at a hemizygous locus. In this regard, it is of note that we were unable to find 2, 6-diaminopurine resistant (DAP^r) PBL's in 2 individuals in another study even though the frequency of TG^r PBL's was greatly elevated in one. This, too, is consistent with the mutation hypothesis since adenine phosphoribosyltransferase (APRT), which is required to phosphorylate DAP, is specified by an autosomal gene (chromosome 16) [44].

Purine analogue resistance in mammalian cells may, of course, be due to factors other than HG-PRT deficiency [45, 46]. Mutation or other alterations at several sites may give rise to TG^r PBL's and current studies are underway to better define the nature of these cells. However, the reasons for resistance may not be strictly relevant to environmental mutagenesis studies if TG^r PBL V_f changes correlate with known mutagen or carcinogen exposures. This correlation has been suggested for cancer patients receiving cytotoxic agents which possess a known capacity to damage the genetic material [21]*.

In the current study, TG^r PBL V_f's in the small group of concurrent control individuals did not differ from those determined over approximately a 2-yr interval for a much larger group of healthy individuals. Psoriatic patients, however, presented definite elevations in the frequencies of these variant cells. These V_f elevations did not depend on whether or not the psoriatic patient was receiving PUVA treatments. By contrast, although TG^r PBL V_f's in vitiligo patients receiving PUVA

treatments were elevated over controls, the V_f's in untreated vitiligo patients did not differ from those of normal individuals.

The finding of elevated TG^r PBL V_f's in psoratic patients receiving PUVA treatments is not unexpected. The human diploid fibroblast system, which assays the same indicator of mutation, is one of the several systems which have shown PUVA to be mutagenic *in vitro* [15]. Since exposure of all lymphocytes in the peripheral blood of man could theoretically be achieved with an 11 min exposure to UVA [18], there is ample opportunity for lymphocytes *in vivo* to receive doses similar to those given to the fibroblasts. That the treated psoriatic and treated vitiligo patient groups both showed elevations of TG^r PBL V_f's suggests that the PUVA itself is responsible.

Psoriatic patients receiving conventional therapy also showed elevations of TG^r PBL V_f's and did not differ from the PUVA-treated group in this regard. All conventionally-treated psoriatic patients had severe psoriasis.

This finding raises the possibility that the psoriatic process itself may result in an increased frequency of TG^r variant lymphocytes. It is known that immunological stimuli are mutagenic to mammalian cells [47,48]. In fact, lymphoid cells may be highly mutable, and this characteristic may be intrinsic to their normal functions [49] and, conversely, may result in a variety of benign and malignant disorders. Immunological mechanisms have been implicated in the pathogenesis of psoriasis [50]. If the psoriatic process itself, for any reason, results in elevated TG^r PBL V_f's, elevations should be seen also in patients not receiving therapy. Studies of untreated individuals with psoriasis are currently in progress.

An alternative possibility exists that some of the therapies employed in the non-PUVA treated psoriatics were themselves mutagenic. Genetic damage to somatic cells could then have resulted if adequate exposure to target cells was achieved. Further investigations are required to evaluate this possibility.

The finding of elevated TG^r PBL V_f's in humans receiving PUVA treatments is not at variance with the finding that sister chromatid exchanges (SCE's) are not elevated in PBL's of these patients under conditions of clinical PUVA use [19]. SCE's may measure a different kind of genetic damage. Also, because SCE's could be induced by PUVA *in vitro*, it is possible that genetic damage occurring *in vivo* does not persist long enough to demonstrate SCE's *in vitro*. Furthermore, there may be selection *in vivo* against damaged cells. Elsewhere, we have presented evidence that there is, in fact, selection *in vivo* against the LN TG^r PBL's [21].* Since TG^r PBL V_f's in normal individuals are not age-related, there also is apparent *in vivo* selection against the non-LN variety of this cell. Nonetheless, recovery of TG^r PBL's resulting from gene mutations may be greater than is the recovery of cells that have experienced the sort of genetic event that allows the demonstration of SCE's.

The current study employs an assay directed towards detecting somatic cell mutations occurring *in vivo* in man. Mutagenesis studies using the several available systems to test isolated agents *in vitro* are important as initial screening tests to identify putative mutagens [1]. However, some agents so identified may have benefits that cannot easily be rejected. In this regard *in vivo* studies have several important features. They may allow an assessment of the genetic effects of these agents under actual conditions of use. They may be used to determine the genetic effects of exposures to specific agents in combination with others. Also, *in vivo* studies are required to assess the importance of pharmacokinetic and metabolic factors that influence the delivery of genetically active agents to genetic targets [1]. Finally, unique individual susceptibilities to genetic damage or the influence of disease in producing such damage may be detected by *in vivo* studies.

The current study suggests the need for longitudinal determinations of TG^r PBL V_f's in psoriatic patients and others receiving PUVA treatments as well as in psoriatic patients receiving conventional therapies. These are underway. Also, TG^r PBL V_f's are being determined in patients with psoriasis who are not on therapy. Our findings to date in PUVA-treated humans are consistent with findings from several sources that suggest that this treatment produces genetic damage. We report them now because of their intrinsic importance, and to present the TG^r PBL autoradiographic assay for consideration as a potential monitoring system to detect somatic cell mutations occurring *in vivo* in man.

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Announcement

The National Institute on Aging invites applications for research grant support in gerontological and geriatric dermatology. Skin problems of the aging and elderly contribute markedly to decline in quality of life. Neither the basc mechanisms responsible for age-associated changes in skin, nor the means for intervention, treatment and/or prevention of the associated problems are adequately understood. Research is necessary at the molecular, cellular and clinical levels. The traditional NIH research grant mechanism and procedures are available to applicants. Application receipt dates for project grants are November 1, March 1, and July 1.

Inquiries are welcome. Potential applicants should notify the NIA with a letter of intent. Address correspondence to: Basic Aging Program—Dermatology, Biomedical Research and Clinical Medicine, Building 31, Room 5C21, National Institute on Aging, NIH, Bethesda, Maryland 20205.